

The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 146. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 146. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 146, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ ID NO: 146, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 146, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 146, or a fragment thereof.

SEQ ID NO: 146 also contains an open reading frame comprising SEQ ID NO: 147. The invention includes a polypeptide comprising SEQ ID NO: 147. SEQ ID NO: 147 is set forth below.

**SEQ ID NO: 147**

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTDQLFLP  
FYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVMNNSTN  
VVIRACNFELCDNPFFAVSKPMGTQHTMIFDNFNCFFEYISDAFSLDVSEKSGNFKHL  
REFVFKNKDGLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDI  
WGTSAAAYFVGYLKPTTFMLKYDENGITIDAVDCSQNPLAELKCSVKSFEDKGIYQTS  
NFRVVPBGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFST  
FKCYGVSATKLNLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPPDDFMGCVL  
AWNTRNIDATSTGNVNYKYRYLRHGKLRPFERDISNVPFSPDGKPCPPALNCYWPLND  
YGFYTTTGIGYQPYRVVLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLT  
SSKRFQPFQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQDV  
NCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYH  
TVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMY  
ICGDSSTECANLLQYGSFCTQLNRALSGIAAEQDRNTREVFAQVKQMYKTPTLKYFGGF  
NFSQILPDLKPTKRSFIEDLLFNKVTADAGFMKQYGECLGDINARDLCAQKFNGLTVL  
PPLLTDDMIAAYTAALVSGTATAGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYEN  
QKQIANQFNKAISQIQESLTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAIISSVLNDI  
LSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRV  
DFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVVFVNG  
TSWFITQRNFFSPQIITDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTS  
PDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGF  
AGLIAIVMVTILLCCMTSCCCLKGACSCGSCCKFDEDDSEPVKGVKLHYT

The invention includes a polypeptide comprising an amino acid sequence having sequence identity to SEQ ID NO: 147. The invention includes a fragment of a polypeptide comprising SEQ ID NO: 147. The invention includes a diagnostic kit comprising a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. The invention includes a diagnostic kit comprising a polynucleotide sequence encoding SEQ

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ID NO: 147, or a fragment thereof. The invention includes an immunogenic composition comprising a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. The invention includes an antibody which recognizes a polypeptide comprising SEQ ID NO: 147, or a fragment thereof. SEQ ID NO: 147 demonstrates functional homology to a coronavirus spike protein.

Predicted transmembrane regions of SEQ ID NO: 147 are identified below.

**Predicted Transmembrane helices of SEQ ID NO: 147**

The sequence positions in brackets denominate the core region.  
Only scores above 500 are considered significant.

Inside to outside helices : 18 found

from	to	score	center
1 ( 1) 16 ( 16)		959	9
233 ( 237) 257 ( 252)		905	244
345 ( 347) 364 ( 361)		490	354
345 ( 354) 369 ( 369)		420	362
497 ( 497) 513 ( 513)		239	506
573 ( 573) 588 ( 588)		811	580
645 ( 648) 666 ( 663)		302	656
690 ( 696) 714 ( 711)		428	704
857 ( 860) 882 ( 874)		1508	867
1031 (1031) 1046 (1046)		446	1039
1199 (1203) 1219 (1217)		2667	1210

Outside to inside helices : 13 found

from	to	score	center
1 ( 1) 17 ( 17)		684	10
222 ( 222) 240 ( 237)		238	229
244 ( 247) 264 ( 264)		613	254
349 ( 355) 369 ( 369)		314	362
496 ( 496) 511 ( 511)		488	503
573 ( 573) 591 ( 591)		712	581
650 ( 652) 666 ( 666)		474	659
674 ( 679) 702 ( 696)		190	686
691 ( 696) 713 ( 711)		210	704
866 ( 868) 886 ( 886)		1172	876
1198 (1201) 1215 (1215)		3221	1208

SEQ ID NO: 147, the spike protein, is a surface exposed polypeptide.

Recombinant expression of a protein can be hindered by hydrophobic transmembrane regions. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 147 wherein one or more of the hydrophobic regions identified above is removed. The invention further includes a polynucleotide encoding such a polypeptide. The invention includes recombinantly expressing the protein in a host cell.

Further characterization of SEQ ID NO: 147 is set forth below.

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PSORT --- Prediction of Protein Localization Sites

version 6.4 (WWW)

MYSEQ 1255 Residues

Species classification: 4

\*\*\* Reasoning Step: 1

Preliminary Calculation of ALOM (threshold: 0.5)

count: 2

Position of the most N-terminal TMS: 496 at i=2

MTOP: membrane topology (Hartmann et al.)

I(middle): 503 Charge difference(C-N): 1.0

McG: Examining signal sequence (McGeoch)

Length of UR: 13

Peak Value of UR: 3.28

Net Charge of CR: 0

Discriminant Score: 8.66

GvH: Examining signal sequence (von Heijne)

Signal Score (-3.5): 5.94

Possible cleavage site: 13

>>> Seems to have a cleavable N-term signal seq.

Amino Acid Composition of Predicted Mature Form:

calculated from 14

ALOM new cnt: 1 \*\* thrshld changed to -2

Cleavable signal was detected in ALOM?: 0B

ALOM: finding transmembrane regions (Klein et al.)

count: 1 value: -12.26 threshold: -2.0

INTEGRAL Likelihood = -12.26 Transmembrane 1202 -1218 (1194 - 1228)

PERIPHERAL Likelihood = 0.16

modified ALOM score: 2.55

>>> Seems to be a Type Ia membrane protein

The cytoplasmic tail is from 1219 to 1255 (37 Residues)

Rule: vesicular pathway

Rule: vesicular pathway

Rule: vesicular pathway

(14) or uncleavable?

Gavel: Examining the boundary of mitochondrial targeting seq.

motif at: 14

Uncleavable? Ipos set to: 24

Discrimination of mitochondrial target seq.:

positive ( 2.18)

Rule: vesicular pathway

Rule: vesicular pathway

Rule: vesicular pathway

\*\*\* Reasoning Step: 2

KDEL Count: 0

Checking apolar signal for intramitochondrial sorting

(Gavel position 24) from: 1 to: 10 Score: 8.0

SKL motif (signal for peroxisomal protein):

pos: 964(1255), count: 1 SRL

SKL score (peroxisome): 0.1

Amino Acid Composition Tendency for Peroxisome: 1.37

AAC not from the N-term., score modified

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Peroxisomal proteins? Status: notclr  
AAC score (peroxisome): 0.079  
Amino Acid Composition tendency for lysosomal proteins  
score: 0.39 Status: notclr  
GY motif in the tail of typeIa? (lysosomal)  
Checking the amount of Basic Residues (nucleus)  
Checking the 4 residue pattern for Nuclear Targeting  
Checking the 7 residue pattern for Nuclear Targeting  
Checking the Robbins & Dingwall consensus (nucleus)  
Checking the RNA binding motif (nucleus or cytoplasm)  
Nuclear Signal Status: negative ( 0.00)  
Type Ia is favored for plasma memb. proteins  
Checking the NPXY motif..  
Checking the YXRF motif..  
Checking N-myristoylation..

## ----- Final Results -----

plasma membrane --- Certainty= 0.460(Affirmative) < succ>  
microbody (peroxisome) --- Certainty= 0.171(Affirmative) < succ>  
endoplasmic reticulum (membrane) --- Certainty= 0.100(Affirmative) < succ>  
endoplasmic reticulum (lumen) --- Certainty= 0.100(Affirmative) < succ>

SEQ ID NO: 147 appears to have a N-terminus signaling region, followed by a surface exposed region, followed by a transmembrane region followed by a C-terminus cytoplasmic domain region. Accordingly, the invention includes an immunogenic, surface exposed fragment of SEQ ID NO: 147. Preferably, said fragment comprises an amino acid sequence which does not include the last 50 amino acids of the C-terminus of SEQ ID NO: 147. Preferably, said fragment comprises an amino acid sequence which does not include the last 70 amino acids of the C-terminus of SEQ ID NO: 147. Preferably, said fragment does not include a transdomain region of SEQ ID NO: 147. Preferably, said fragment does not include a C-terminus cytoplasmic domain of SEQ ID NO: 147. Preferably, said fragment does not include a N-terminus signal sequence. Preferably, said fragment does not include amino acids 1 – 10 of the N-terminus of SEQ ID NO:147. Preferably, said fragment does not include amino acids 1 – 14 of the N-terminus of SEQ ID NO: 147.

The spike protein of coronaviruses may be cleaved into two separate chains into S1 and S2. The chains may remain associated together to form and form a dimer or a trimer. Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 147 wherein said polypeptide has been cleaved into S1 and S2 domains. The invention further includes a polypeptide comprising SEQ ID NO: 147 wherein amino acids 1 – 10,

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preferably amino acids 1 – 14 of the N-terminus are removed and further wherein SEQ ID NO: 147 is cleaved into S1 and S2 domains. Preferably the polypeptide is in the form of a trimer.

Predicted N-glycosylation sites of SEQ ID NO: 147 are identified below:

**Prediction of N-glycosylation sites of SEQ ID NO: 147**

SeqName	Position	Potential	Jury agreement	NGlyc result
SEQID 147	29 NYTQ	0.7751	(9/9)	+++
SEQID 147	65 NVTG	0.8090	(9/9)	+++
SEQID 147	109 NKSQ	0.6081	(7/9)	+
SEQID 147	119 NSTN	0.7039	(9/9)	++
SEQID 147	158 NCTF	0.5808	(7/9)	+
SEQID 147	227 NITN	0.7518	(9/9)	+++
SEQID 147	269 NGTI	0.6910	(9/9)	++
SEQID 147	318 NITN	0.6414	(9/9)	++
SEQID 147	330 NATK	0.6063	(8/9)	+
SEQID 147	357 NSTF	0.5746	(8/9)	+
SEQID 147	589 NASS	0.5778	(6/9)	+
SEQID 147	602 NCTD	0.6882	(9/9)	++
SEQID 147	699 NFSI	0.5357	(7/9)	+
SEQID 147	783 NFSQ	0.6348	(9/9)	++
SEQID 147	1080 NGTS	0.5806	(7/9)	+
SEQID 147	1116 NNTV	0.5106	(5/9)	+
SEQID 147	1176 NESL	0.6796	(9/9)	++

Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the glycosylation sites identified above. The invention further includes a polynucleotide encoding one or more of the fragments identified above. This glycosylation site can be covalently attached to a saccharide. Accordingly, the invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the glycosylation sites identified above and wherein said polypeptide is glycosylated at one or more of the sites identified above.

Predicted O-glycosylation sites are identified below:

**Prediction of O-glycosylation sites**

Name	Residue No.	Potential	Threshold	Assignment
SEQID 147	Thr 698	0.8922	0.7696	T
SEQID 147	Thr 706	0.9598	0.7870	T
SEQID 147	Thr 922	0.9141	0.7338	T
SEQID 147	Ser 36	0.8906	0.7264	S
SEQID 147	Ser 703	0.8412	0.7676	S

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the o-glycosylation sites identified above. The invention further includes a polynucleotide encoding one or more of the fragments identified above. The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the O-glycosylation sites identified above and further wherein the polypeptide is covalently bonded to a saccharide at one or more of the included glycosylation sites.

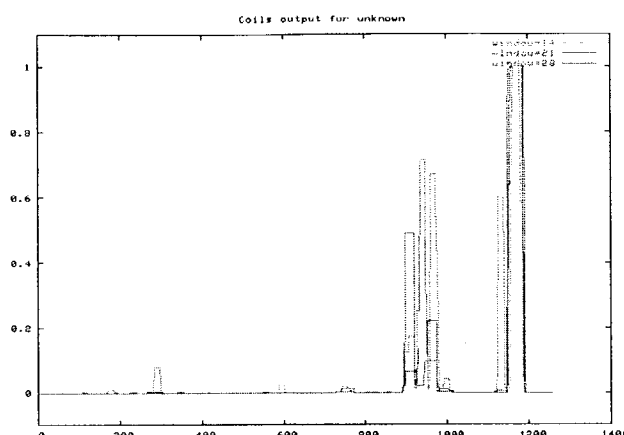
The invention further includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises one or more of the N-glycosylation sites identified above and further wherein said fragment comprises one or more of the O-glycosylation sites identified above.

The invention includes a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment does not include one or more of the glycosylation sites identified above. The invention also includes a polynucleotide encoding such a polypeptide.

Predicted phosphorylation sites of SEQ ID NO: 147 are Ser-346, Tyr-195, and Tyr-723. Accordingly, the invention comprises a polypeptide comprising a fragment of SEQ ID NO: 147 wherein said fragment comprises at least ten amino acid residues and wherein said fragment comprises one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723. In one embodiment, one or more of the amino acids selected from the group consisting of Ser-346, Tyr-195, and Tyr-723 are phosphorylated.

Predicted coiled coils of SEQ ID NO: 147 are identified below:

Coiled coil Prediction:



Accordingly, the invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 147 wherein said fragment includes a coiled region of SEQ ID NO: 147. The invention comprises a polypeptide sequence comprising a fragment of SEQ ID NO: 147, wherein said fragment does not include a coiled region of SEQ ID NO: 147.

The ORF1a and ORF1b sequences of coronaviruses are typically translated as a single ORF1ab polypeptide. Slippage of the ribosome during translation generates an a-1 frameshift. One region of such slippage is illustrated below:

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gggttttacacttagaacaacagtcctgtaccgctctgcggaatgtggaaagggttatggctgtagttgtga
+1  G F T L R N T V C T V C G M W K G Y G C S C D
+3  G F Y T - K H S L Y R L R N V E R L W L - L -
    ccaactccgcgaacccttgatgcagtcctgcggatgcatcaacggttttaaacggggttgcggtgtaagt
+1  Q L R E P L M Q S A D A S T F L N G F A V - V
+3  P T P R T L D A V C G C I N V F K R V C G V S
    gcagcccgctttacaccgtgcggcacaggcactagtactg
+1  Q P V L H R A A Q A L V L
+3  A A R L T P C G T G T S T

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which would generate the following translational slippage:

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ccaactccgcgaacccttgatgcagtcctgcggatgcatcaacggttttaaacggggttgcggtgtaagt
  Q L R E P L M Q S A D A S T F L N R V C G V S

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Accordingly, the invention includes a polypeptide comprising SEQ ID NO: 148. SEQ ID NO: 148 is set forth below.

#### SEQ ID NO: 148

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MESLVLGVNEKTHVQLSLPVLQVRDVLVRGFGDSVEEALSEAREHLKNGTCGLVELEKGVLPQLEQPYV
FIKRS DALSTNHCHKVVELVAEMDGIQYGRSGITLGLVLPVPHVGETPIAYRNVLLRKNNGKAGGHSYGI
DLKSYDLGDELGTDPIDYEQNWN TKHGSGALRELTRELNGGAVTRYVDN NFCGPDGYPLDCIKDFLAR

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